# Solid-Phase Radiopharmaceutical Precursors: Rapid Preparation of Iodine Labelled Phenylpiperazinium Ions from a Polymer-Supported Stannane

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**Abstract**

To serve as a precursor to N-4-[$^{122}$I]iodophenyl-N', N'-dimethylpiperazinium ion, a polymer-supported 4-stannylphenylpiperazinium acetate has been prepared and characterized by MAS $^{119}$Sn NMR spectroscopy. Reaction with iodine established a loading capacity of 1.3 mmol of phenylpiperazinium ion/g of polymer. Varying either the amount of resin, 0.5-5 mg (60 s reaction time), or the reaction time, 15-300 s (5 mg resin), resulted in consistent radioiodination yields >97%. Under optimized conditions the resin was reacted for 15 s with Na $^{125}$I and peracetic acid followed by filtering through an anion exchange resin to give N-4-[$^{125}$I]iodophenyl-N', N'-dimethylpiperazinium ion in >90% radiochemical yield and >99% radiochemical purity with a specific activity of 1,950 Ci/mmol and a total tin concentration of <100 ppb.

**Keywords:** polymer-supported stannane, radioiodine, iodine-122, solid phase reagent

## 1. Introduction

Myocardial perfusion scintography is one of the most widely applied imaging methods for the assessment of perfusion defects associated with coronary artery disease. The current clinical studies are performed almost exclusively with SPECT imaging and the single photon agents $^{201}$Tl, $^{99m}$Tc-sestamibi or $^{99m}$Tc-tetrofosmin. Even though generator produced $^{82}$Rb is commercially available and cyclotron produced $^{13}$N-ammonia has recently been approved for perfusion studies, PET perfusion imaging has not yet gained prominence. Quantitative PET and PET/CT perfusion imaging would provide a significant improvement over current clinical practice.

Recent efforts in our laboratory have been aimed at developing iodine-122 labelled quaternary ammonium salts for PET perfusion imaging.[1] Iodine-122 is a generator produced 3.63 min positron emitter with a 78% positron branch ($E_{\beta+}$ = 3.2 MeV) produced by the decay of its 20 hour parent isotope xenon-122. This generator system was first developed at Brookhaven National Laboratory[2] and applied at Lawrence Berkeley National Laboratory[3] with $^{122}$Xe from Crocker Nuclear Laboratory cyclotron[4] at the University of California, Davis. The short-lived iodine offers several advantages for perfusion imaging including the possibility for rapid sequential imaging studies, performing multiple tracer studies within a short time frame, the convenience of a generator system, and the potential to tap into the rich iodine chemistry to produce labeled compounds with suitable biological activity. The short half-life does, however, present some unique challenges for the preparation of injectable radiotracers.

There are three generally applied methods for producing iodine labelled radiopharmaceuticals, direct iodination, radioiodine-for-$^{127}$I exchange and iododemetallation. All of these methods have been utilized to produce $^{122}$I labelled tracers[5-9], however, each method

is not without shortcomings. The direct iodination method provides non-specifically labelled compounds and it is not uncommon for more than one regioisomer to be produced. This would necessitate a potentially time-consuming purification process, such as HPLC separation, to be utilized. The biggest drawback of the radioiodine-for-$^{127}$I exchange reaction is the preparation of low specific activity radiotracers. The most common iododemetallation reactions employ aryltrialkylstannane precursors. The aryltrialkylstannane and trialkylstannyl side products resulting from the electrophilic iodination reaction must be removed from the final solution as organotin compounds are known to be toxic.[10, 11] In all three cases the separation of the reagents, unreacted precursors and by-products from the desired radiopharmaceutical presents the stiffest challenge to the timely and efficient preparation of the iodine labelled probes.

The successful application of polymer-supported stannanes for the preparation of [$^{123}$I]- and [$^{131}$I]-MIBG has been reported.[12] The general strategy for polymer-supported stannane precursors, illustrated in Figure 1, commences with an "attachment" step in which a polymer-supported chlorostannane **1** is reacted with an aryl-lithium to produce a polymer-supported arylstannane intermediate. In subsequent "modification" steps the desired polymer-supported radiopharmaceutical precursor can be prepared. Reagents are used in excess relative to the polymer in order to drive the reactions to completion. Isolation and purification of the polymer-bound product is then simply achieved by washing away excess reagents and reaction by-products.

With the polymer-supported tin precursor in hand, the radiopharmaceutical can be released from the polymer backbone on demand using the appropriate radiohalide (*$F^-$, *$Br^-$, *$I^-$ or *$At^-$) and oxidant. Unlike the preparation of the polymer-supported precursor where the polymer is used as the limiting reagent, the radiolabeling step sees the polymer in excess. In

using the radionuclide as the limiting reagent only the desired radioactive product is released from the polymer allowing for simple isolation and purification. This radiolabeling procedure provides high specific activity radiopharmaceutical in high yield with high chemical and radiochemical purity. Additionally, one achieves regioselective incorporation of the radiohalogen into the desired radiotracer with all potentially toxic tin species remaining bound to the polymer that is readily removed from the solution. Given the widespread use of radioiododestannylation reactions one can envision the potential utility of the polymer-supported stannyl precursors.

The purpose of the present study is to determine if the polymer supported stannyl precursor could be used for the rapid and efficient labeling of radiopharmaceuticals with iodine-122. While initial accounts of the application of the polymer-supported MIBG precursor for iodine-123 and iodine-131 labeling reported reaction times as long as 2 hours,[12] subsequent reactions have been performed in less than 15 minutes, still too long for iodine-122. However, the stannyl phenylpiperizinium precursor should be strongly activated towards electrophilic aromatic substitution and, thus, be more reactive. Taken together these observations encouraged us to believe that the reaction of the radioiodide with the precursor and subsequent purification of the radiotracer would be consistent with the use of the short-lived iodine-122 for the preparation of radiochemically pure, high specific activity PET probes.

## 2. Materials and Methods

### 2.1 *General*

Reagents and solvents were purchased from Aldrich, Fisher Scientific or BDH, Inc. and were used without further purification. The identity and purity of precursors was verified by $^{1}$H-NMR spectroscopy. Anhydrous THF was freshly distilled from a still with a potassium/ benzophenone indicator. The low resolution FAB samples were run on a VG 70-SE (Manchester, UK) equipped with a FAB source operated at 8 kV. The high resolution FAB samples were run on a VG ZAB2-EQ (Manchester, UK) also equipped with a FAB source; electron impact mass spectra on a Finnigan-MAT 8200 mass spectrometer and UV-visible spectra using a Varian Cary 100 Bio UV-visible spectrometer. The solution $^{1}$H NMR and $^{13}$C NMR spectra were obtained using either a Varian Gemini 300 (300 MHz for $^{1}$H) or a Varian Mercury 400 (400 MHz for $^{1}$H) spectrometer; the solid phase MAS $^{119}$Sn NMR spectra were obtained on a Varian Infinity Plus 400 spectrometer (400 MHz for $^{1}$H) using a 7.5 mm probe spinning at an angle of 54.74°. The spectra were obtained using a simple pulse and acquire, with a 45° pulse and a 5 sec delay between pulses. In addition, the polymers were pre-treated with toluene prior to obtaining the MAS $^{119}$Sn NMR spectra. HPLC were run using a Varian Vista 5500 controller, Shimadzu SPD-10A UV-visible detector, Dionex 4400 integrator and a μBondpak C-18, 4.6 x 250 mm cartridge column. 'Purified' water was at the 18 Mohm level. HPLC buffer solutions (pH = 7.4) were 5 mM in sodium dihydrogenphosphate and 5 mM in disodium hydrogenphosphate. The N-4-bromophenylpiperazine **2** and N-4-iodophenylpiperazine **3** were prepared following published procedures.[13] The poly-3- and poly-4-(2-di-*n*-butylchlorostannyl)ethylstyrene-*co*-divinylbenzene, **1**, was prepared as previously

described.[14] Quantitative tin analyses were obtained from ToxScan Inc., Watsonville, CA using ICP/MS and EPA method 200.8.

## 2.2 *Chemistry*

### 2.2.1 *N-4-Bromophenyl-N'-methylpiperazine* (**4**)

A solution of **2** (4.80 g, 20 mmol), 95 % formic acid (4.5 mL, 100 mmol) and aqueous 37 % formaldehyde (3.3 mL, 44 mmol) was held at reflux under argon for 18 h. After the reaction mixture was cooled, concentrated hydrochloric acid (1.0 mL, 27 mmol) was added and the liquids were removed on the rotary evaporator. The resultant solid was dissolved in methylene chloride which was washed with excess dil. NaOH, three times with water. After drying, the methylene chloride was evaporated to yield a pale yellow solid. Extraction of this solid with hot hexanes followed by evaporation of the hexanes gave **4** as a white solid (2.75 g, 54 %) of mp. 55-56$^{o}$C. $^{1}$H-NMR spectrum ($CDCl_3$) δ, ppm: 2.37 (3H, s), 2.59 (4H, m), 3.19 (4H, t), 6.78 (2H, AA' of an AA'BB'), 7.32 (2H, BB' of an AA'BB'), $^{13}$C NMR spectrum ($CDCl_3$) δ, ppm: 46.19, 48.98, 54.97, 111.63, 117.46, 131.67, 150.07, HRMS m/z: Calculated for $C_{11}H_{15}N_2{}^{81}Br$ = 254.042, observed = 254.042

### 2.2.2 *N-4-Iodophenyl-N'-methylpiperazine* (**5**)

To a stirred solution of **3** (1.33 g, 4.6 mmol) and Proton-Sponge® (0.99 g, 4.6 mmol) in acetonitrile (25 mL) was added methyl iodide (0.65 g, 4.6 mmol) in 15 mL of acetonitrile over a period of 6 h. The resultant precipitate was collected by filtration, dissolved in methylene chloride and extracted with dil. NaOH. The dichloromethane layer was washed with water, dried with anhydrous sodium sulphate and evaporated to yield a slightly yellow solid. A TLC (10%

MeOH: 90% $CHCl_3$) of the solid showed the presence of Proton-Sponge®. Two successive column chromatographies, collecting the early eluting fractions, served to remove the proton sponge impurity. Recrystallization from methanol/water yielded **5** (700 mg, 2.3 mmol, 50%) of a white solid. $^1$H-NMR spectrum ($CDCl_3$) δ, ppm: 2.37 (3H, s), 2.68 (4H, m), 3.18 (4H, m), 6.69 (2H, AA' of an AA'BB'), 7.50 (2H, BB' of an AA'BB'). $^{13}$C NMR spectrum (DMSO-$d_6$) δ, ppm: 47.54, 50.18, 54.90, 83.21, 117.12, 138.41, 149.21

2.2.3 *N-4-Iodophenyl-N', N'-dimethylpiperazinium iodide* (**6**)

Methyl iodide (735 μL, 12 mmol) and **5** (360 mg, 1.2 mmol) were combined in 1 mL of acetonitrile in a capped vial. After 2 h, the precipitate was collected by filtration and washed several times with acetonitrile to yield **6** as a white solid (180 mg, 0.41 mmol, 54 %) of mp. 245-246$^o$C. $^1$H NMR spectrum (DMSO-$d_6$) δ ppm: 3.2 (6H, bs), 3.5 (8H, bs), 6.81 (2H, bd), 7.53 (2H, bd). $^{13}$C NMR spectrum (DMSO-$d_6$) δ, ppm: 41.54, 50.28, 59.90, 82.21, 118.05, 137.44, 148.99; HRMS m/z: Calculated for $C_{12}H_{18}N_2I$ = 317.051, observed = 317.051

2.2.4 *Poly-N-2-(3-{dibutyl[2-(3-and 4-vinylphenyl)ethyl]stannyl}phenyl)-N'-methylpiperazine-co-divinylbenzene* (**7**)

Under a flow of argon, 20 mL of freshly distilled dry THF was added by syringe to **4** (270 mg, 1.1 mmol) in a three-necked 100 mL round-bottom flask, equipped with a T-bore stopcock, a rubber septum and a powder addition side arm containing 466 mg of chlorostannane polymer **1** (~ 0.70 mmol). The flask and its contents were outgased three times at dry ice/acetone temperatures and an argon atmosphere was introduced. To this solution, n-butyllithium (530 μL, 1.1 mmol, 2.5 M) was added dropwise at –78°C, with the resultant formation of a yellow color.

After 2 h at –78°C, the polymer was tipped into the THF solution, and the suspension was allowed to stir for 18 h and warm slowly to RT. To the suspension, about 3 mL of methanol was added and the suspension was filtered. The solid was washed with methanol, water, methanol/water/acetone, methanol/acetone and methanol several times to yield 500 mg of **7**. MAS $^{119}$Sn NMR spectrum (toluene) δ ppm: -42.2

2.2.5 *Poly-N-2-(3-{dibutyl[2-(3-and 4-vinylphenyl)ethyl]stannyl}phenyl)-N', N'-dimethylpiperazinium iodide-co-divinylbenzene* (**8a**)

Polymer **7** (160 mg) and methyl iodide (68 μL, 1.1 mmol) were combined in 1.5 mL of acetonitrile in a capped vial. After 4h of shaking, the contents of the vial were collected by filtration and washed with several portions of methanol, water and acetone to yield 150 mg of **8a** after air drying. MAS $^{119}$Sn NMR spectrum (toluene) δ ppm: -41.1

2.2.6 *Poly-N-2-(3-{dibutyl[2-(3-and 4-vinylphenyl)ethyl]stannyl}phenyl)-N', N'-dimethylpiperazinium acetate-co-divinylbenzene* (**8b**)

Approximately 1 g of polymer **8a** in a sintered glass filter funnel was washed 6 times with 2 mL of 1 M sodium acetate in 70% aqueous ethanol followed by ten 95% ethanol rinses of 2 mL each.

2.2.7 *General iodinolysis procedure*

To a suspension of polymer **8b** (27 mg) in 0.5 mL of acetonitrile was added 1.5 mL (0.15 mmol) of a 0.1 M solution of iodine in acetonitrile. After gentle stirring for 2 h, an excess of aqueous 0.1 M sodium thiosulphate was added. The solution was transferred to a 25 mL

volumetric flask and diluted to the mark with methanol. HPLC analysis (flow rate = 2 mL/min, eluent concentration 50% methanol: 50% pH =7.4 buffer, and λ=250 nm) of a filtered portion showed a peak at 41.5 min attributed to **6** by comparison/coinjection with an authentic sample.

This procedure was utilized to determine the loading capacity (mmol of **5** per gram of polymer **7** or mmol of **6** per gram of either polymers **8a** or **8b)**.

2.2.8 *N-4-Tributylstannylphenyl- N'-methylpiperazine* (**9**)

To **4** (975 mg, 3.8 mmol) in 15 mL of THF at -78$^{o}$ C under an argon atmosphere was added n-BuLi (1.66 mL, 3.8 mmol) in a dropwise manner. After being allowed to stir at –78$^{o}$ C for 30 minutes, tributylchlorostannane (1.04 mL, 3.8 mmol) was added dropwise and the solution was allowed to warm to room temperature overnight. Removal of the THF under vacuum yielded a light yellow oil (1.89 g). TLC, $^{1}$H NMR spectroscopy and HPLC indicated successful formation of **9**. However significant impurities were also present. A 30 mg portion was purified by 6 x 5 mg preparative HPLC runs (flow rate = 7 mL/min, eluent 100% methanol, and λ=254 nm) of the crude product dissolved in methanol to yield **9** (11 mg) as an oil eluting at 11 minutes accounting for 58 % of the total area. $^{1}$H-NMR spectrum ($CDCl_3$) δ, ppm: 0.85 (9H, t), 0.97 (6H, t), 1.30 (6H, m), 1.50 (6H, m), 2.32 (3H, s), 2.57 (4H, t), 3.19 (4H, t), 6.90 (2H, AA' of an AA'BB'), 7.32 (2H, BB' of an AA'BB'). $^{13}$C NMR spectrum ($CDCl_3$) δ, ppm: 9.33, 13.54, 27.23, 28.94, 46.01, 48.46, 54.99, 115.50, 130.34, 137.08, 150.80; HRMS m/z: Calculated for $C_{23}H_{43}N_2{}^{118}Sn$ = 465.244, observed = 465.244

2.2.9 *N-4-Tributylstannylphenyl- N', N'-dimethylpiperazinium iodide* (**10**)

To an unpurified sample of **9** (180 mg, 0.39 mmol) in 4 mL of acetonitrile in a sealed vial was added methyl iodide (36 μL, 0.58 mmol) and reaction was stirred for 24 h at room temperature. After removal of the solvent under vacuum a yellow solid was obtained. Recrystallization from a small volume of methanol produced **10** as a white solid (90 mg, 0.19 mmol) in 33% yield; mp. 162-164$^{o}$C. $^{1}$H-NMR spectrum (methanol-$d_4$) δ, ppm: 0.90 (9H, t), 1.15 (6H, t), 1.37 (6H, m), 1.55 (6H, m), 3.31 (6H, s), 3.57 (4H, bs), 3.66 (4H, bs), 7.05 (2H, AA' of an AA'BB'), 7.38 (2H, BB' of an AA'BB'). $^{13}$C NMR spectrum (DMSO-$d_6$) δ, ppm: 9.10, 13.60, 26.70, 28.65, 41.66, 50.26, 60.13, 115.60, 129.94, 136.89, 149.30; HRMS m/z: Calculated for $C_{24}H_{45}N_2{}^{120}Sn$ = 481.260, observed = 481.261

*2.3 Radiochemistry*

2.3.1 *General*

High specific activity Na[$^{125}$I]iodide in pH 8-12 aqueous 0.01M NaOH was purchased from Amersham Biosciences. RadioHPLC was performed using a Waters 590 solvent pump with an in-line Linear™ Model UV-106 UV detector (254 nm) an in-line radioactivity detector (Carroll and Ramsey Associates, Model 105S).

2.3.1 *Preparation of the hydrogen peroxide/acetic acid oxidant*

Glacial acetic acid (1 mL) and 30% hydrogen peroxide (1.67 mL) were combined in a foil covered 25 mL volumetric flask and left overnight at room temperature. After dilution to the mark with 'purified' water, the solution was kept cold. Just prior to radiolabelling, a small

portion of the solution was transferred to an amber vial for immediate use. The oxidant was made fresh for each radiolabelling experiment.

2.3.2 *N-4-[$^{125}$I]Iodophenyl-N', N'-dimethylpiperazinium ion* ([**$^{125}$I]-6**)

To a 4 mL glass vial with stirbar was added polymer **8b** (0.5-5 mg), ethanol (200 μL), 0.1 M potassium dihydrogen phosphate (200 μL). The vial was set aside for 10 minutes to allow for swelling of the polymer. A refrigerated portion of the hydrogen peroxide-acetic acid solution was transferred to a 4 mL amber glass vial and along with the polymer reaction solution was placed in a glove box. Into the polymer reaction vial, Na$^{125}$I (4 μL, 350 μCi) was added followed quickly by hydrogen peroxide-acetic acid solution (40 μL). After 30 sec, sodium meta-bisulphite solution (20 μL of a 10 % by weight solution) was added followed by an excess of a saturated sodium bicarbonate solution. The reaction solution was filtered through a 0.45 μm nylon filter cap into a clean 4 mL glass vial. To the reaction mixture was added 'purified' water (200 μL) and following a final filtration the vial contained 329 μCi of material. The filtered solution was analyzed by reversed phase HPLC (Waters μBondapak C18 4.6 x 250 mm, flow rate = 1 mL/min, mobile phase: 65% methanol: 35% (1% $NEt_3$ in $H_2O$ adjusted to pH=7.4 with $H_3PO_4$) and λ=254 nm). The filtered aqueous solution was passed through a small column containing 75 mg of moistened anion exchange beads (BioRad, AG I-X8 hydroxide form) over a period of 30 s. An aliquot of the processed solution was analyzed by reversed phase HPLC. In subsequent reactions the filter was not used and the crude reaction mixture was passed (filtered) directly through the anion exchange column.

## 3.0 Results and Discussion

### 3.1 *Chemistry*

As illustrated in Scheme 1, the polymer-supported arylstannane precursor **8b** was synthesized in four steps from 4-bromophenylpiperazine **2**. Piperazine **2** was monomethylated using the Eschweiler-Clarke procedure[15], chosen in order to avoid the observed quaternization when direct methylation with methyl iodide was used. The tertiary amine **4** was converted to its monolithio derivative by reaction with an equivalent of n-butyllithium at -78$^{o}$ in THF. This ratio of reagents was chosen so that there would be little or no n-butylithium remaining to compete with the monolithio derivative of **4** for the Sn-Cl sites on the polymer. The aryllithium intermediate was added to the chlorostannane polymer **1** in amounts chosen such that the monolithio intermediate would be in 1.5-2 fold excess. This ratio of reagents was chosen in an attempt to drive the coupling reaction at the Sn-Cl sites on the polymer to completion.

Polymer **7** was characterized by MAS $^{119}$Sn NMR spectroscopy and by reaction with an excess of iodine (iodinolysis). The $^{119}$Sn NMR spectrum showed one peak at -42.2 ppm. This is a significant shift from that observed for the Sn-Cl of polymer **1** at +143 ppm and is consistent with other examples of polymer-supported arylstannanes produced in our laboratories. The observation of one peak at -42.2 ppm and the lack of a peak remaining at + 143 ppm confirmed the complete conversion of polymer **1** to polymer **7**. The loading capacity of polymer **7** was determined by HPLC measurement of the amount 4-iodophenyl-methylpiperazine **5** produced after iodinolysis. The loading capacity was 1.3 mmol of **5** released per gram of polymer **7**. Standard solutions for calibration of the HPLC were prepared from an authentic sample of **5** which was synthesized by monomethylation of 4-iodophenylpiperazine **3** with methyl iodide as

shown in Scheme 2. As mentioned above, this reaction resulted in some quaternization product that was chromatographically separated from the desired monomethylated piperazine **5**.

Treatment with an excess of methyl iodide was used to convert polymer **7** into polymer **8a** (Scheme 1) which was analyzed by MAS $^{119}$Sn NMR spectroscopy and by iodinolysis. The $^{119}$Sn NMR spectrum showed one peak at -41.1 ppm. This small shift difference (-41.1 vs –42.2 ppm) in $^{119}$Sn NMR peaks does not allow for unequivocal conclusion on the extent of conversion of polymer **7** to polymer **8a**. However, the HPLC examination of the iodinolysis reaction shows only quaternary 4-iodophenylpiperazinium iodide **6** with little or no 4-iodophenyl-methylpiperazine **5**. Standard solutions for calibration of the HPLC were prepared from an authentic sample of **6** which was synthesized by methylation of N-(4-iodophenyl)-N'-methylpiperazine **5** (Scheme 2). Additionally, the loading capacity for polymer **8a** of 1.3 mmol/g was identical to the starting polymer **7**. Taken together the NMR and iodinolysis results confirm the quantitative conversion of polymer **7** to polymer **8a**.

In order to maximize specific activity during radioiodination, the iodide counterion of polymer **8a** was exchanged for acetate by several successive washes with sodium acetate giving polymer **8b**. The success of this treatment was established during the radiolabelling process by measurement of the specific activity of the [$^{125}$I]**6** (discussed below).

As shown in Scheme 3, the aryltributylstannyl analogs were also prepared using a process paralleling the polymer route in Scheme 1. The 4-bromophenylpiperazine **4** was converted to the corresponding 4-tributylstannyl phenyl analog **9** by formation of the monolithio analog of **4** and reaction with tributyltin chloride. Subsequent alkylation with methyl iodide gave the stannyl piperazinium iodide **10**. This precursor allows a comparison between radiolabeling with the polymers and the solution counterparts.

3.2 *Radiochemistry*

All radiolabelling experiments were carried out using iodine-125 and polymer **8b,** acetate form, in order to avoid dilution of the radioiodide and to work as close as possible to carrier free concentrations. Dilute peracetic acid (hydrogen peroxide/acetic acid) was chosen as the oxidant to simplify the purification procedure and since it had proven to be effective in the case of the polymer-supported MIBG precursor.[12] The reaction time and mass of polymer-bound precursor were varied in order to find the minimum tolerable value for both parameters.

The radiosynthesis was remarkably straightforward. The polymer precursor was allowed to swell in a solution of ethanol and 0.1M potassium dihydrogen phosphate. The desired quantity of $Na^{125}I$, typically less than 500 μCi, was added to the swollen polymer solution followed immediately by the peracetic acid solution. The reaction was quenched at the desired time by addition of sodium meta-bisulphite. Initially, the reaction mixtures were filtered to remove the solid polymer material. An HPLC analysis of this filtrate, shown in Figure 2A, produced two peaks in the radioactivity trace: one at the solvent front (ranging for 3-5%) attributed to $^{125}$I-iodide and one at 7.05 min (97%) confirmed to be [$^{125}$I]**6** by coinjection of authentic **6**. In order to remove the remaining unreacted $^{125}$I-iodide from the solution the filtrate was passed through a minimal quantity of anion exchange resin. This eliminated the unreacted $^{125}$I-iodide, as shown in the HPLC trace (Figure 2B), with minimal loss of total radioactivity, less than 10%. Thus, the overall radiochemical yield for the labeling and purifcation was greater than 90%. In order to increase the efficiency of the product purification, the separate initial filtration step was removed and the ion exchange resin bed was used to filter out the solid polymer material. The total time for the purification process was reduced to less than 30 s.

The effect of reaction time, 15 s to 5 min, on radiochemical yield and radiochemical purity was investigated. All of the reactions were carried out using ~5 mg of the precursor polymer **8b**. The reactions were timed from the addition of the peracetic acid to the addition of the excess reductant. The total radioactivity after filtration was determined and an aliquot of the filtrate was analyzed by radioHPLC. The results of these experiments are presented in Figure 3. The percent of the product in solution was determined by HPLC and does not represent isolated yields.

As seen in Figure 3, down to the shortest reaction time that could be reasonably achieved, 15 s, the conversion of radioiodide to [$^{125}$I]**6** was essentially complete, greater than 98 %. Superimposed on Figure 3 is the effect of simulated iodine-122 decay on the yields with increasing time. Given the high yield at such a short reaction time makes this approach attractive for use with the short-lived iodine-122. It was reasonable to anticipate that polymer **8b** would be one of the more reactive polymer-supported arylstannanes since the amino group in the 4-position should be strongly activating towards electrophilic substitution at the stannyl ipso position.

The role of the amount of polymer **8b** on radiochemical yield was also determined under typical reaction conditions. The results of these reactions with precursor masses ranging from 0.5 to 5 mg are presented in Figure 4. The conversion of $^{125}$I-iodide to [$^{125}$I]**6** was essentially complete in every case. In each of these reactions the radioidide is, by far, the limiting reagent. The 1.3 mmol/g loading capacity of polymer **8b** translates into 1.3 μmol of the stannane available per 1 mg of **8b**. By comparison, 350 μCi of iodine-125 corresponds to 0.16 nmol of radioiodide. In the reaction of 1 mg of polymer **8b** with 350 μCi of iodine-125 the ratio of reagents is nearly 5 orders of magnitude with respect to the polymer-supported precursor. Thus,

only a very small fraction of the polymer-supported precursor is consumed. In principle polymer **8b** could be reacted again but there seems to be little benefit in investigating this possibility given the small quantity of polymer **8b** actually needed in an individual radiolabelling procedure.

Quantification of the UV peak on the HPLC of the final product with standards prepared from **6** yielded a specific activity of 1,950 Ci/mmol which is close to the theoretical maximum of 2,175 Ci/mmol. This serves to establish that the 6 washes of polymer **8a** with 1M acetate produced polymer **8b** with little iodide remaining. Given that the $^{125}$I-iodide is the limiting reagent and that the free iodine has been removed by successive washings of the polymer, it is reasonable to project that the specific activity of the radiopharmaceuticals produced using the polymer-supported precursor would be as high as that of the starting radionuclide.

3.3 *Tin Analysis*

One of the proposed benefits of using polymer-supported arylstannanes for the production of radiopharmaceuticals is the avoidance of tin impurities in the final product. This hypothesis was tested by determining the total concentration of tin in the reaction products of material prepared using Na$^{127}$I at concentrations mimicking the commercial iodine-125 (~40 μM) employed here. In each case the reaction mixture was either filtered or passed through an anion exchange column and then diluted to 1 mL with water. The resultant solution was analyzed for total tin by ICP/MS with a detection limit of 5 ppb. The results of these reactions are presented in Table 1 and, with the exception of entry 3, are the average of four reactions.

Entries 1 and 2, both using 5 mg of polymer **8b** for 30 s, compare the effect on the tin concentration of purifying the reaction mixture by either filtration or by direct use of an ion exchange column. Both methods show that similar, low levels of tin are being released into solution under the radiolabelling conditions. A comparison of reaction times of 30 s and 60 s

(entries 2 and 3) indicate that longer reaction times result in higher levels of tin. Likewise, a comparison of reaction times of 60 and 300 s with 1 mg of polymer **8b** (entries 4 and 5) again show an increase in tin levels but not in the same proportions as with entries 2 and 3. When the amount of polymer **8b** is decreased to 1 mg from 5 mg (entries 3 and 4) the amount of tin released is approximately halved. The general trend is that the amount of tin in the reaction solutions is decreased by reducing the amount of polymer **8b** and by decreasing the reaction time. Since the results presented in Figures 3 and 4 indicate that reaction times as short as 15 s and amounts of polymer **8b** as little as 0.5 mg can be used in radiolabelling, it should possible to keep tin contamination at a very low level. In comparison to entry 2, the amount of tin is much larger when the free tributylstannyl analog, **10**, is reacted under the same conditions, entry 6. Not only was there a greater concentration of the tin species in the solution but significant post-reaction purification is needed.

In summary, we have demonstrated that polymer-supported precursors can be used to obtain radiodinated radiopharamceuicals at a carrier-free level in high radiochemical yields in a total reaction and purification time of about 1 min. The use of the stannyl solid-phase reagent approach permits regiospecific labeling while minimizing the toxic by-products and reducing the need for time-consuming purification scenarios. Given these attributes, the polymer-supported stannane precursor presents an attractive strategy for use with the 3.63 min iodine-122.

## 4.0 Conclusion

In the present study we have developed a reliable, reproducible method for the rapid and efficient synthesis of radioiodinated phenylpiperazinium ions using a polymer-supported stannane precursor. The polymer-supported precursor can be readily produced by coupling the

desired lithio analog of the phenylpiperazine to the polymer stannyl chloride. Further modification of the polymer-bound precursor to form, in this case, the quaternary amine was quantitative. With as little as 0.5 mg of the polymer, the corresponding radioiodinated phenylpiperazinium ion was produced in high radiochemical yield in as little as 15 s. Filtration of this reproducible reaction through an anion exchange column removed the excess, unreacted radioiodide as well as the excess polymer-supported precursor. With this precursor, high specific activity, radiochemically pure, tin-free N-4-[$^{125}$I]iodophenyl-N',N'-dimethylpiperazinium ion was obtained in about a minute. Analogs of the N',N'-dimethyl piperazinium salt, used herein as a model compound, demonstrating desirable perfusion imaging characteristics may potentially be labelled with the short-lived iodine-122, using the polymer-supported stannane strategy, for PET perfusion studies.

**Acknowledgments**

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**Table 1.**

Table 1. Total tin concentration as a function of precursor mass and reaction time

| Entry | Precursor (mass) | Reaction Time (s) | Purification Method | [Tin][a] (ppb) |
|---|---|---|---|---|
| 1 | **8b** (5 mg) | 30 | Filter | 22 ± 6 |
| 2 | **8b** (5 mg) | 30 | Ion Exchange | 54 ± 26 |
| 3 | **8b** (5 mg) | 60 | Ion Exchange | 215 ± 70[b] |
| 4 | **8b** (1 mg) | 60 | Ion Exchange | 129 ± 44 |
| 5 | **8b** (1 mg) | 300 | Ion Exchange | 205 ± 34 |
| 6 | **10** (5 mg) | 30 | Ion Exchange | 6100 ± 1500 |

[a] Determined by ICP/MS using EPA Method 200.8, n=4

[b] n=2

Figure and Scheme Legends.

Figure 1. Strategy for preparing polymer supported radiopharmaceutical precursors.

Figure 2. HPLC analysis of the iodophenylpiperizinium ion A) after filtering off the polymer and B) after passing through 75 mg AG1X-8 ion exchange resin.

Figure 3. Radioiodination reaction time course, using 5 mg polymer, as measured by HPLC. ♦ Yield measured with iodine-125. ● Calculated overall yield with iodine-122.

Figure 4. Radioiodination yield as a function of the mass of polymer for a 60 s reaction. Yield was measured by HPLC.

Scheme 1. Synthesis of iodinated intermediate (**4)** and N-4-Iodophenyl-N', N'-dimethylpiperazinium iodide (**6**)

Scheme 2. Synthesis of the polymer-supported stannyl precursor and N-4-[$^{125}$I]Iodophenyl-N', N'-dimethylpiperazinium ion (**[$^{125}$I]6**)

Scheme 3. Synthesis of the stannyl precursor N-4-Tributylstannylphenyl- N', N'-dimethylpiperazinium iodide (**10**) and the corresponding radioiodinated N-4-[$^{125}$I]Iodophenyl-N', N'-dimethylpiperazinium iodide (**[$^{125}$I]6**).

**Figure 1.**

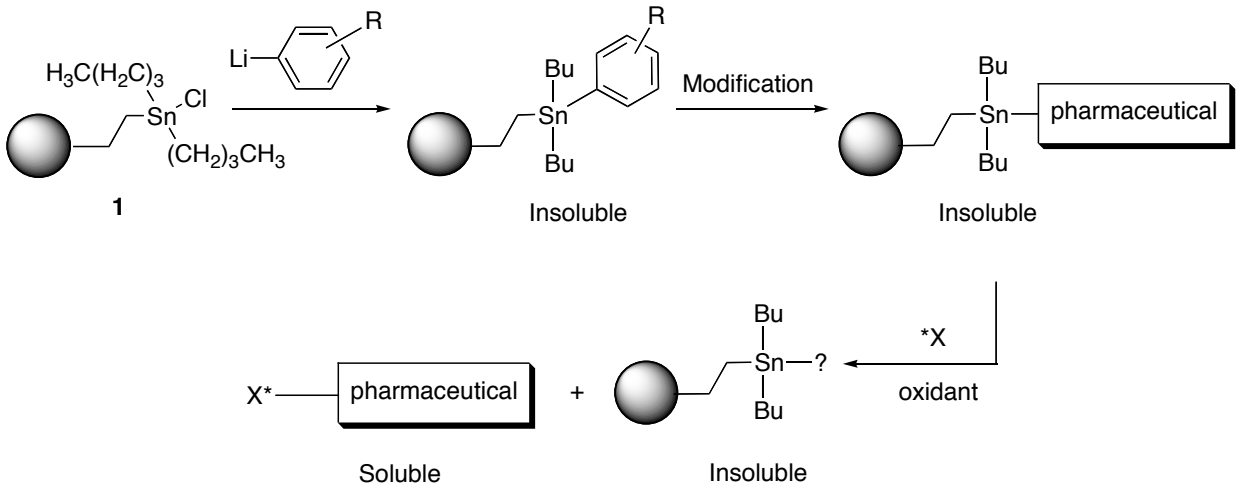

H3C(H2C)3
Sn
Cl
(CH2)3CH3
Li
R
Bu
Bu
Modification
pharmaceutical
1
Insoluble
Insoluble
*X
oxidant
X*
pharmaceutical
+
?
Soluble
Insoluble

**Figure 2.**

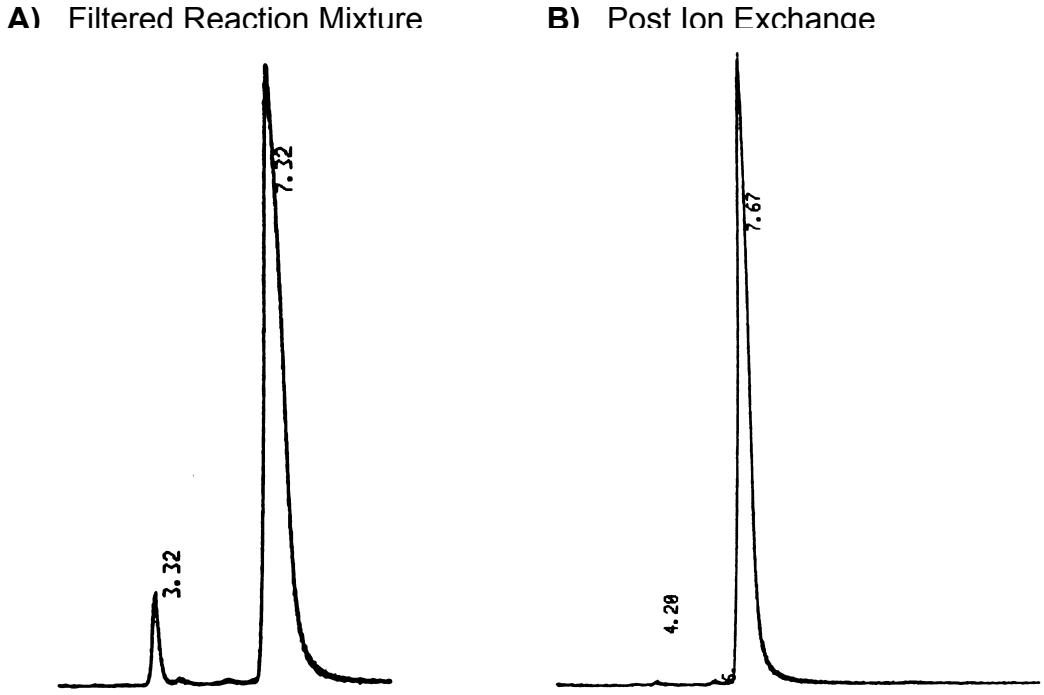

A) Filtered Reaction Mixture
B) Post Ion Exchange
3.32
7.32
4.20
7.67

**Figure 3.**

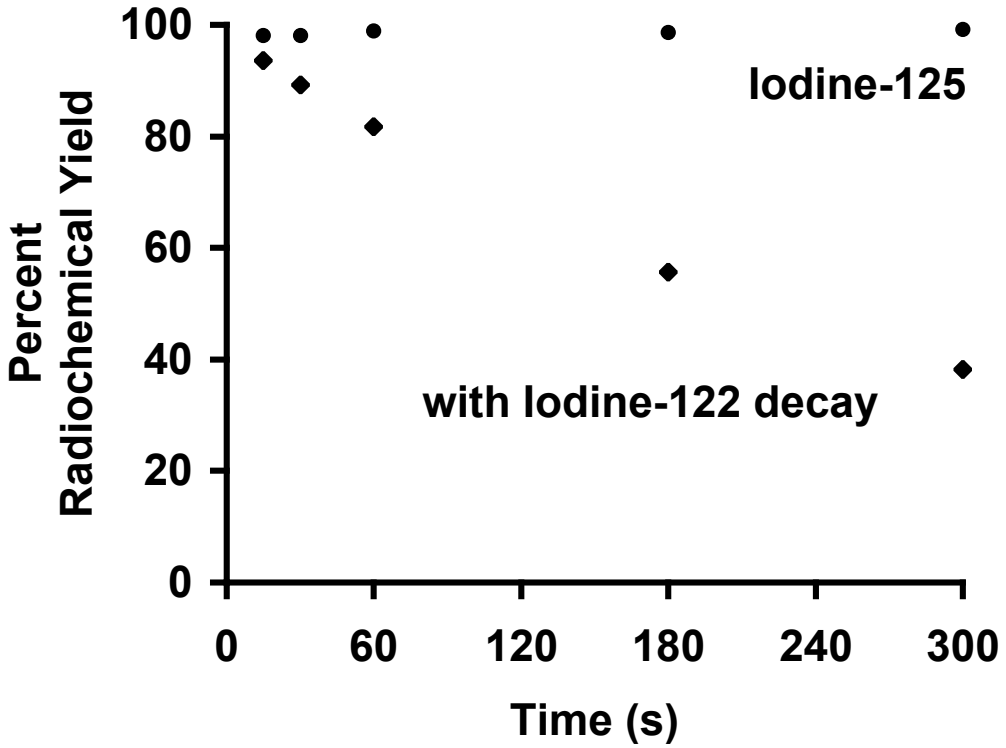
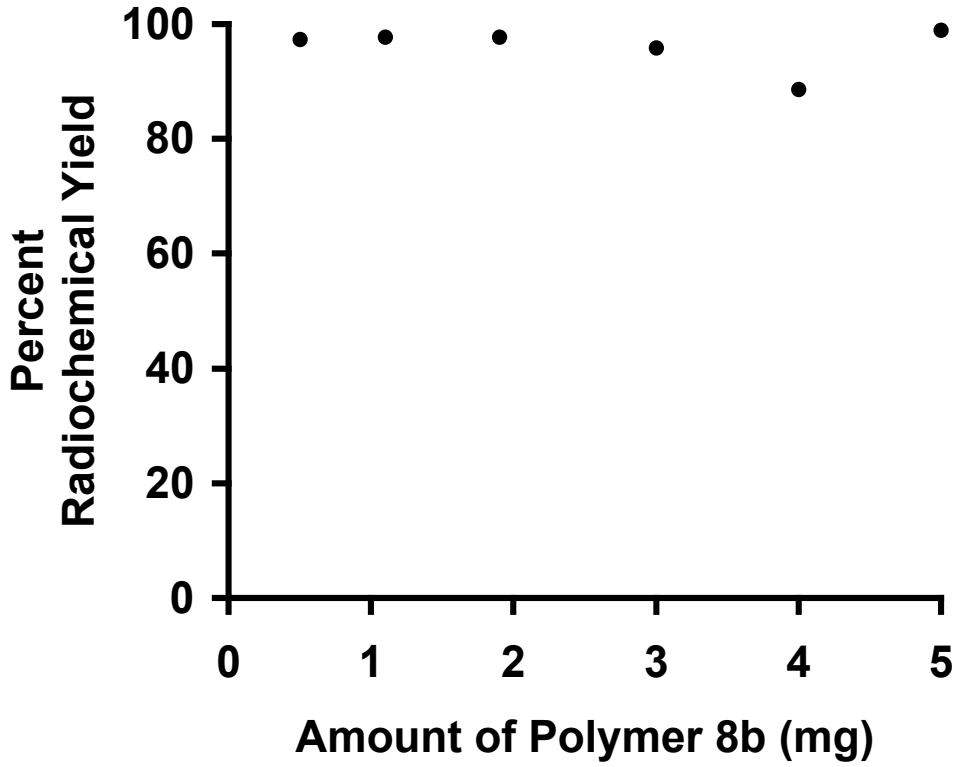
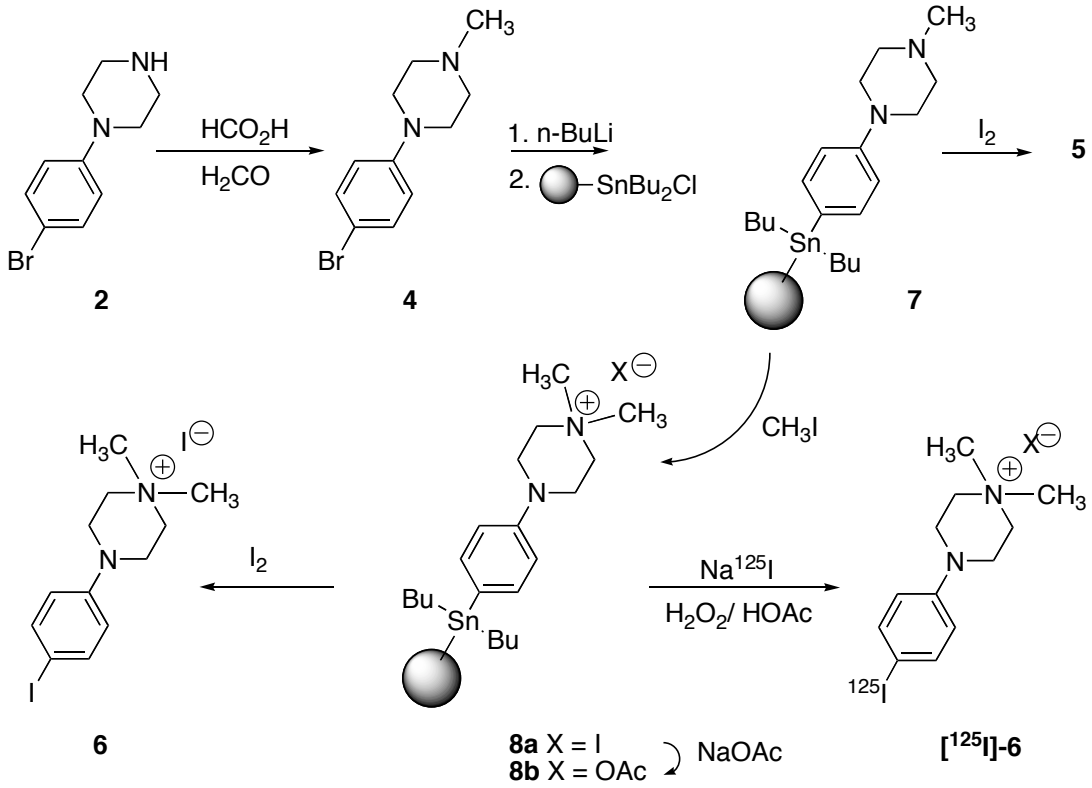

Percent
Radiochemical Yield
100
80
60
40
20
0
0
60
120
180
240
300
Time (s)
Iodine-125
with Iodine-122 decay

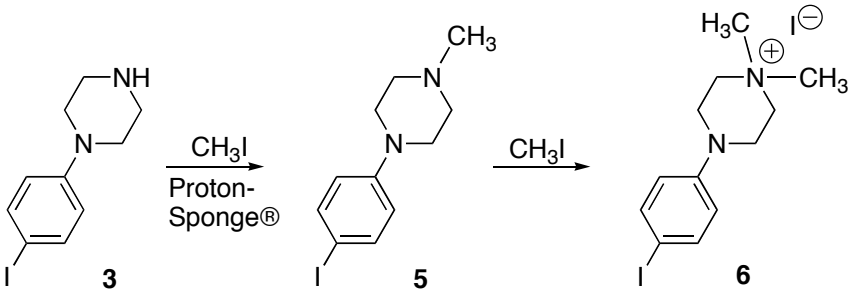
**Figure 4.**


Percent
Radiochemical Yield
100
80
60
40
20
0
0
1
2
3
4
5
Amount of Polymer 8b (mg)

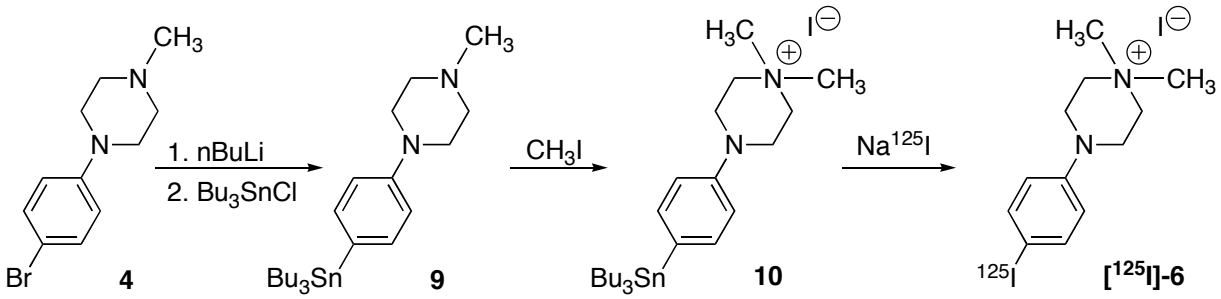
**Scheme 1.**


NH
N
Br
HCO2H
H2CO
CH3
1. n-BuLi
2. SnBu2Cl
Bu
Sn
Bu
I2
5
2
4
7
H3C
X
CH3
CH3I
I
I2
Na125I
H2O2/ HOAc
125I
6
8a X = I
8b X = OAc
NaOAc
[125I]-6

**Scheme 2.**



**Scheme 3.**


CH3
N
N
Br
4
1. nBuLi
2. Bu3SnCl
CH3
N
N
Bu3Sn
9
CH3I
H3C
I⊖
⊕
N
CH3
N
Bu3Sn
10
Na125I
H3C
I⊖
⊕
N
CH3
N
125I
[125I]-6